A Phase I Trial of AAV-Mediated Liver-Directed Gene Therapy for Hemophilia B

Stanford University Medical Center

The Children's Hospital of Philadelphia

Avigen, Inc.

Today's Focus

Issues Pertaining to IGLT of AAV Vector

SUMMARY OF AAV MEDIATED INTEGRATION IN LIVER

- ~5% of hepatocytes are stably modified with rAAV
- The proportion of integrated genomes is small, generally < 10% of double stranded vector DNA
- Gene expression from integrated and episomal AAV genomes parallels the proportion of vector DNA in each state
- There is no detectable increase in the proportion of integrated genomes over time
- The proportion of transduced cells with integrated genomes is small
- Most integrants are one or two copy genomes

Clinical Trial Objectives

- Test the hypothesis that AAV-mediated liver-directed gene transfer is safe.
- Characterize the human immune response to the transgene product and to vector.
- Determine whether germline transmission of vector occurs following hepatic administration.
- Determine dose capable of producing clinically relevant Factor IX levels in the blood.

Clinical Trial Design

Phase I open-label, dose escalation safety trial of AAV-hFIX administration by infusion into the hepatic artery

Vector Administration

- Vector is infused into liver via a balloon occlusion catheter placed in the hepatic artery.
- Factor IX protein will be administered before and following procedure.
- Subjects are observed at least 24 hours

Dose Escalation Plan

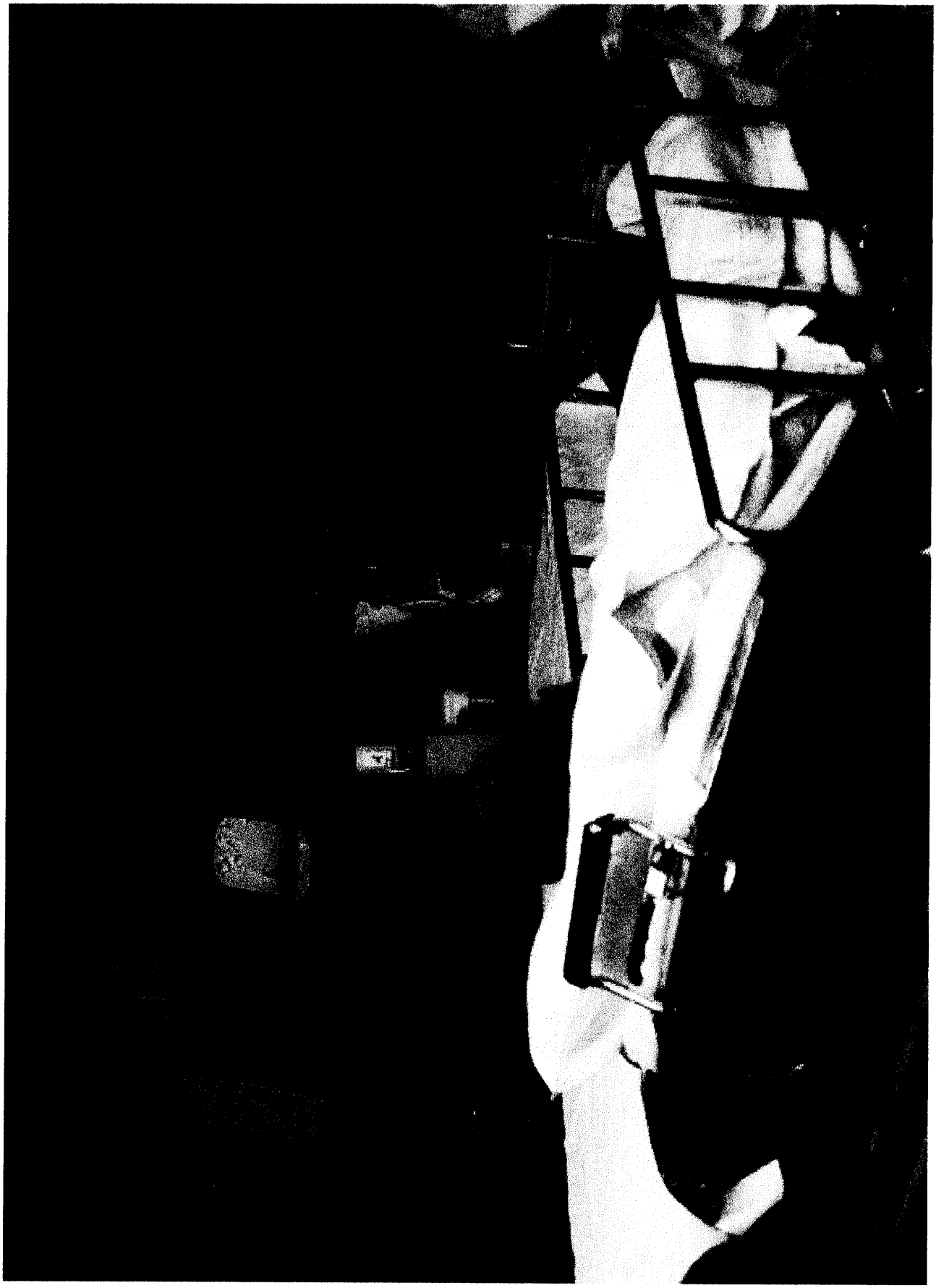
Group	Subjects	Dose/kg[1] (v.g.)[2]	Total Dose[3] (v.g.)	Observed F.IX levels in mice	Observed F.IX levels in dogs
1	2	2.0×10^{11}	1.4×10^{13}	~1%	-
2	4	1.0×10^{12}	7.0×10^{13}	5%	4-12%[4]
3	4	5.0×10^{12}	3.5×10^{14}	20-30%	-

[1] dosing will be performed according to the patient weight obtained at the time of injection
[2] vector genomes
[3] assuming a 70 kg adult
[4] levels observed with a similar, but not identical construct.

New Exclusion Criteria*

- Patients unwilling to provide required semen samples.
- Patients unable to provide semen samples of adequate semen volume ($\geq$1.5mL), sperm count of at least 20 x 10^6 sperm/mL, and motility ($\geq$50%).

*Based on low-volume ejaculates in subject #2



Subject #1

- 63 Y/O male with severe factor IX deficiency
- S/P bilateral knee replacement 5 years PTA
- HIV negative
- HCV positive; HCV viral load negative
- Social Hx: father of 3; grandson with hemophilia

Subject #1

- Procedure (8/13/01)
- Received 2.0 x 10^{11} vector genomes/kg
- No complications
- D/C home to referring HTC after 5 days

Subject # 1: Hematology Data

- All CBCs within normal limits

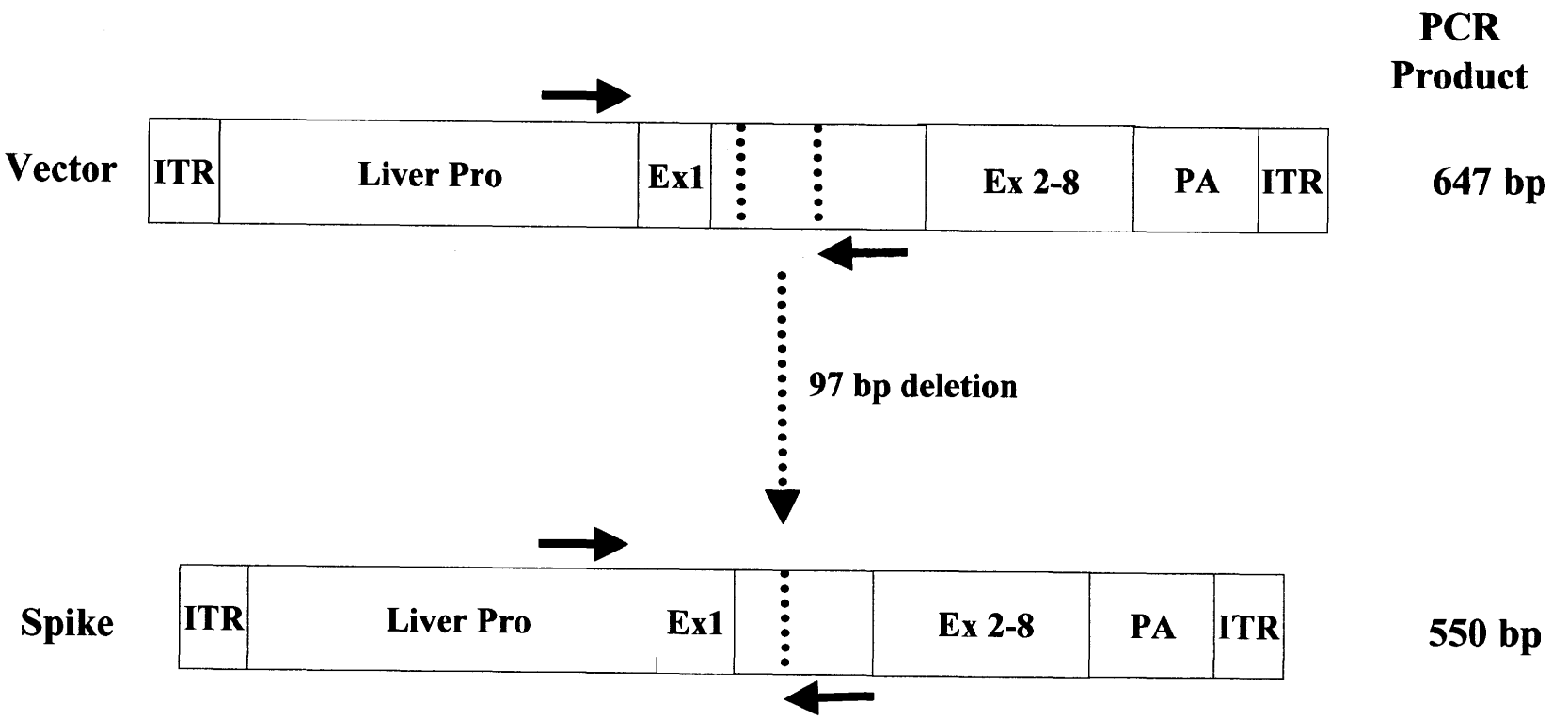
Subject # 1: LFT / PT

- **All liver function tests and prothrombin time within normal limits**

Subject # 1: Coagulation Data

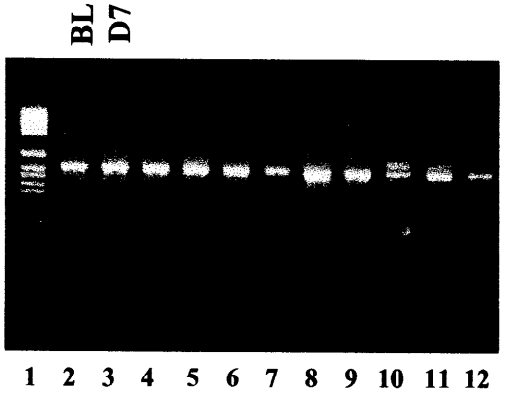
- No therapeutic levels or inhibitors observed

Design of Clinical PCR Assay

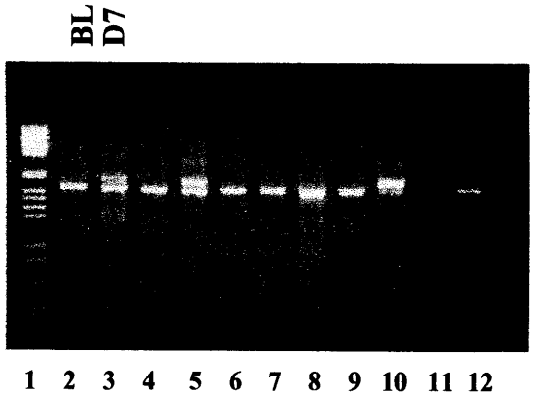


Example of PCR Analysis

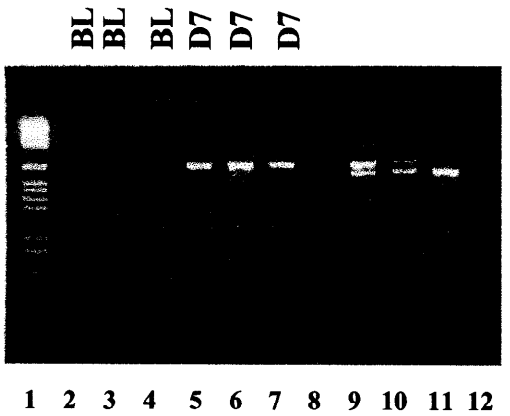
100 copies



10 copies



unspiked



Subject # 1: Detection of Vector Sequences by PCR

Subject # 1: Semen Analysis for Vector DNA

Samples performed in triplicate

*** Week 3 sample fractionation: Motile sperm negative, seminal fluid positive,seminal fluid pellet positive, total semen positive.**

Sensitivity of Assay: less than 1 copy per 30,000 haploid genomes.

Subject # 2

- 48 Y/O male with severe factor IX deficiency
- S/P bilateral knee replacement 1999, bilateral elbow replacement 2001
- HIV positive, HCV positive
- Hx Non-Hodgkin's lymphoma, 1986. Relapse in 1996
- Meds: Viracept, Epivir, Hivid.

Subject # 2

- Procedure (1/29/02)
- Received 2.0 x 10^{11} vector genomes/kg
- No complications
- D/C to referring HTC after 7 days

Subject # 2: LFT / PT

- All liver function tests and prothrombin time within normal limits

Subject # 2: Renal Function

- Renal function within normal limits

Subject # 2: Hematology Data

- **All CBCs within normal limits**

Subject # 2: Coagulation Data

- Factor IX levels?
- No inhibitors observed

*Infusion with exogenous F.IX >14 days prior to FIX assay.

Subject # 2: Detection of Vector Sequence by PCR Analysis

Subject # 2: Semen Analysis for Vector DNA

*.07 mg of DNA extracted from month one sample

Conclusions

- Subjects #1 and #2 tolerated the procedure well
- Vector DNA present transiently in total semen from Subject #1, not present in motile sperm fraction at week 3
- Limited data in Subject #2
- Enrollment of subjects at mid-dose proceeds only if subject #2 shows absence of signal in motile sperm fraction